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The molecular biology of HIV latency: Breaking and restoring the Tat-dependent transcriptional circuit

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Abstract

Purpose of review—Despite the remarkable success of intensive antiretroviral drug therapy in blocking the HIV replication, the virus persists in a small number of cells where HIV has been transcriptionally silenced. This review will focus on recent insights into the HIV transcriptional control mechanisms that provide the biochemical basis for understanding latency.

Recent findings—Latency arises when the regulatory feedback mechanism driven by HIV Tat expression is disrupted. Small changes in transcriptional initiation, induced by epigenetic silencing, can lead to restrictions in Tat levels and entry of proviruses into latency. In resting memory T-cells, which carry the bulk of the latent viral pool, additional restrictions limiting cellular levels of the essential Tat cofactor P-TEFb and the transcription initiation factors NF-κB and NFAT ensure that the provirus remains silenced unless the host cell is activated.

Summary—Strategies to purge the latent proviral pool require non-toxic activator molecules. The multiple restrictions imposed on latent proviruses that need to be overcome suggest that proviral reactivation will not be achieved when only a single reactivation step is targeted, but will require both removal of epigenetic blocks and the activation of P-TEFb. Alternatively, new inhibitors that block proviral reactivation could be developed.

Keywords

HIV Tat; HIV initiation; HIV elongation; HIV chromatin; P-TEFb

Introduction

Highly active antiretroviral therapy (HAART) for HIV infections uses a potent cocktail of antiviral drugs to stably reduce virus replication below detectable levels. Unfortunately, even after decades of treatment, the virus is able to persist and high levels of virus replication invariably resume when treatment is interrupted [1,2]. The main cause of treatment failure appears to be reactivation of virus from a pool of latently infected cells, principally residing in the pool of resting memory CD4+ T-cells [3,4].

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Although it is difficult to exclude the possibility that slowly replicating virus persists in sanctuary sites which are poorly accessed by the antiviral drugs, genetic evidence strongly suggests that this is not the case. Both the residual virus recovered from treated patients [5], and the rebounding virus recovered during the short treatment interruptions [6], have much greater sequence homogeneity than would be expected for a viral population replicating at low levels, implying that both sets of viruses were derived from the reactivation of clones of long-lived latently infected cells.

During latent infections transcription from HIV proviruses is effectively suppressed. This review will describe how recent insights into the molecular mechanisms controlling HIV transcription have defined how HIV becomes initially silenced and then subsequently re-emerges from latency. These molecular studies provide the framework for developing new therapeutics that target the latent viral pool.

Transcriptional feedback: The HIV promoter is bipolar

HIV transcription is tightly controlled both at the level of transcription initiation and elongation. There is no specific host repressor that directs a provirus to become latent. Instead, the switch between productive transcription and latency is due to the manipulation of a powerful feedback mechanism fueled by the viral trans-activator protein, Tat.

The HIV LTR which includes multiple upstream DNA regulatory elements that serve as binding sites for cellular transcription initiation factors. The core promoter, which includes all the elements required for transcription in productive infections, is a powerful and highly optimized promoter comprised of three tandem SP1 binding sites [7], an efficient TATA element [8] and a highly active initiator sequence [9]. SP1 functions as an essential upstream activator; deletion of one or more of the SP1 sites effectively blocks both Tat-dependent and basal transcription [10]. In addition, the HIV LTR carries a unique “enhancer” sequence which contains two tandem NF-κB binding motifs [11]. Members of both the NF-κB family and NFAT can bind to the HIV NF-κB motifs and is stimulated by cooperative interactions with Sp1 [12]. In contrast to the elements of the core promoter, mutation of the NF-κB sites results in only a modest inhibition of virus growth in most cell lines [13], however, signaling through the viral enhancer is essential in order to re-activate latent proviruses.

The key feature that distinguishes the HIV LTR from cellular promoters is that is auto-regulated by Tat. Detailed biochemical investigations over the last 25 years have shown that Tat exclusively stimulates transcription elongation. In the absence of Tat, transcription initiation is normal but only short abortive transcripts are produced. Tat directs the cellular transcriptional elongation factor P-TEFb [14] to nascent RNA polymerases by binding to the HIV TAR element, an RNA stem-loop structure found at the 5′ end of all viral transcripts (Figure 1). P-TEFb is a protein kinase complex comprised of a regulatory Cyclin T1 (CycT1) subunit and the catalytic CDK-9 subunit. This enzyme potently stimulates HIV elongation by targeting both positive and negative elongation factors. Phosphorylation of the negative elongation factor NELF removes a powerful block to elongation [15], while phosphorylation of the C-terminal domains (CTD) of RNAP II [16] and Spt5 [17,18] results in the direct activation of polymerase processivity.

In the culmination of over 2 decades of research on P-TEFb by David Price and his colleagues, the crystal structure of a Tat:pTEFb complex was determined earlier this year [19••]. The structure shows that Tat forms extensive contacts both with the CycT1 subunit of P-TEFb and also with the T-loop of the Cdk9 subunit. Importantly, Tat induces significant conformational changes in CDK9 providing an explanation for how it is able to constitutively activate the enzyme [19••].

The strong amplification of transcription stimulated by Tat, coupled with the disproportionate decline in transcription that ensues when Tat levels become restricted, gives the HIV promoter a “bipolar” character (Figure 2). Insightful studies by Weinberger et al. [20,21] and Burnett et al. [22•] have emphasized how stochastic fluctuations in Tat gene expression can act as a molecular switch. Small changes in initiation rates, which can be experimentally mimicked by introducing mutations into the NF-κB and Sp1 binding sites, are sufficient to restrict Tat production and lead to enhanced rates of viral entry into latency [22•]. This switching mechanism crucially depends on the auto-regulation of Tat. When Tat is expressed in trans from an ectopic promoter, HIV proviruses become constitutively active and are unable to enter latency [23].

The idea that the switch between active transcription and latency is regulated by Tat expression levels is also supported by the observation that introduction of mutations that attenuate Tat activity leads to an increased frequency of viruses entering latency [23]. Similarly, viruses recovered from the latently-infected CD4+ T cells of patients are enriched for HIV-1 Tat variants with impaired transactivation activity [24].

In summary, changes in the cellular environment that restrict transcription initiation are able to reduce Tat availability and force the virus into latency, but the virus remains poised to resume its replication in response to triggers that stimulate transcription initiation and restore Tat levels.

How is this subtle balance achieved?

Epigenetic silencing and promoter occlusion: Triggering the depression

When HIV infects cells it preferentially integrates into active transcription units that provide a favorable environment for viral transcription [25]. An early hypothesis to explain latency was that latently infected cells were generated by rare integrations into heterochromatic regions [26]. However extensive sequencing studies have shown that latent proviruses are almost invariably found integrated into actively transcribed genes [27-29]. This implied that following the initial integration and expression of the proviruses epigenetic silencing events restricted HIV expression. In support of this hypothesis, numerous studies have shown that the LTRs of latent proviruses acquire heterochromatic structures (Figure 3). Typically latent proviruses display a fixed nucleosomal structure that blocks the transcription start site [30], high levels of histone deacetylases (HDACs), deactylated histones [31-33] and methylated histones [23,34•,35-36]. The functional importance of these modifications is clearly demonstrated by the observation that drugs that inhibit histone deacetylation or methylation are potent inducers of latent proviruses [37,38].

Two recent reports have demonstrated that the acquisition of hypermethylated CpG islands near the HIV promoter correlates with the silencing of HIV transcription in both Jurkat T-cells and primary isolates aviremic patients [39•,40•]. Treatment of latently infected cells with the DNA methylation inhibitor 5-aza-deoxycytidine (5-aza-CdR) potentiates the reactivation and outgrowth of silenced proviruses [39•,40•]. Typical clonal cell lines carrying a single latent proviruses show heterogenous levels of methylated histones and methylated DNA. This suggests that silencing is an ordered and progressive process, with DNA methylation repressing one of the most repressed states.

The observation that HIV can integrate within active transcription units prompted investigations into whether latency arises because of transcriptional interference between the host promoter and the viral LTR. Lenasi et al. [41] showed that in latently infected Jurkat T-cell lines, host-initiated transcripts terminated at the polyadenylation site in the 5’ LTR of the integrated proviruses. Similarly, Han et al. [42] showed in cell lines that were engineered

to insert HIV proviruses in either orientation downstream of the HPRT gene, readthrough transcription inhibited HIV-1 gene expression for convergently orientated provirus but enhanced HIV-1 gene expression when HIV-1 was in the same orientation as the host gene.

Although transcriptional interference was clearly documented in both these studies, it remains unclear whether it is a primary cause of latency or a consequence of the insertion of a repressed provirus into an active gene. Duvinger et al. [43•] have argued that transcriptional interference leads to the “silent integration” of proviruses in the majority of latently infected cells. However, these experiments were designed to select for population of viruses that were subjected to immediate silencing events. By contrast, studies from our laboratory have shown that after the selection of cells that carry highly expressed viruses there is progressive silencing due to the imposition of epigenetic restrictions [23,34•].

In the natural setting, epigenetic silencing is imposed when HIV infects T-cells just prior to their natural reversion to a quiescent state during the differentiation of both naïve and memory T-cells [3,44,45]. Importantly, these silencing events can also be recapitulated in vitro using primary cells that are induced to enter quiescence [34•,40,46-48]. It therefore seems likely that both transcriptional interference and epigenetic silencing can contribute to the development of latency in vivo. The net effect of both mechanisms is to restrict proviral transcription initiation and induce a decline in Tat levels.

Active restriction of HIV transcription elongation: Deepening the depression

In contrast to transformed cells, resting CD4+ T-cells further ensure that latent proviruses remain transcriptionally inactive by imposing additional powerful blocks that restrict P-TEFb levels [49]. In these cells, the majority of the essential elongation factor P-TEFb is sequestered into a large RNP complex comprising 7SK RNA and a series of RNA binding proteins (7SK snRNP) [50,51]. Essential components of the 7SK snRNP complex include HEXIM1 or HEXIM2, which inhibit the CDK9 kinase in a 7SK-dependent manner [52,53].

The inactivation of P-TEFb effectively prevents any basal transcriptional activation by Tat-independent recruitment of P-TEFb to the provirus. For example, we have observed that induction of latently infected primary CD4+ T-cells requires T-cell receptor mediated induction of P-TEFb [34•]. Although the mechanisms leading to P-TEFb release from the 7SK snRNP during T-cell activation are not yet fully defined, it seems likely that post-translational modifications of the complex are required. For example, the HIV activator HMBA is able to induce dephosphorylation of the T-loop of P-TEFb by PP1a and PP2B resulting in its release from the 7SK snRNP [54]. An important recent study suggests that cyclin T1 acetylation also triggers dissociation of HEXIM1 and 7SK RNA from the inactive 7SK snRNP complex and activates the transcriptional activity of P-TEFb [55•].

Unpublished studies from our laboratory have shown that the restriction of elongation from the HIV LTR from latent proviruses is much stronger than for cellular promoters. This is due to powerful additional restrictions on HIV elongation which are imposed by NELF [15,56,57]. Furthermore, we have shown that depletion of NELF provides a potent signal to reactivate latent proviruses.

Transcription initiation and chromatin remodeling: Dispelling the lassitude

In addition to restricting P-TEFb, quiescent T-cells also sequester the cellular transcription initiation factors NF-κB and NFAT in the cytoplasm [11,58]. TCR stimulation induces a complex cascade of pathways leading to the activation of NF-κB through a protein kinase C-

mediated pathway and NFAT through the Ca^{+2}-calcineurine pathway. Recent structural studies have shown that both NF-κB [59] and NFAT [60] assume unique, mutually exclusive, conformations upon binding the HIV LTR.

NF-κB binding to the HIV LTR triggers proviral reactivation by directing recruitment of the histone acetyltransferases (HATs) to the HIV LTR [61-64]. The acetylation of histones near the HIV promoter in turn provides a signal for the recruitment of the chromatin remodeling complex BAF which activates transcription by displacing the restrictive nucleosome-1 (Nuc-1) positioned immediately downstream from the transcriptional start site [65]. The recruitment of HATs may also help to stabilize NF-κB on the viral promoter, since acetylation [66] and methylation [67] of the p65 subunit enhances its affinity its DNA binding affinity. NFAT also interacts with the HIV LTR via the NF-κB binding sites, it seems likely that members of the NFAT family also promote chromatin remodeling of the HIV-1 since they are known to recruit the coactivators p300 and CBP to cellular genes [68].

There has been a long standing controversy about whether NFAT [46,69,70] or NF-κB [34•, 71] is the dominant factor mediating proviral reactivation in $CD4^+$ T-cells. This seems to be an unnecessary distinction because it is now evident that multiple cellular pathways are able to independently reactivate latent HIV expression. In latently infected primary T-cells derived from thymocytes both the PKC pathway leading to NF-κB activation and the NFAT are able to stimulate virus expression [71]. TLR5 stimulation induces activation of NF-κB and can reactivate latent HIV-1 in quiescent central memory CD4+ T cells [72]. Similarly, inducers of NF-κB such as prostratin and HIV-1-reactivating protein factor (HRF) [73] are potent inducers of latent HIV proviruses in resting memory T-cells. By contrast, in polarized T-cells generated *in vitro*, NFAT is clearly the exclusive activator of latent proviruses [46].

Tat, P-TEFb and the positive control of HIV elongation: Mania

When proviruses emerge from latency, the initial rounds of HIV transcription are restricted until the resumption of new Tat synthesis after about 2 hrs [23,74]. Our picture of how Tat and P-TEFb stimulate HIV elongation has been radically altered by two recent papers which used modern proteomic methods to discover two distinct and stable complexes containing Tat and P-TEFb [75••,76••] (Figure 4). The most abundant complex contains active P-TEFb and the human transcription factors/coactivators AFF4, ENL, AF9, and ELL2. He et al. [75••] showed that ELL2, an elongation factor previously shown to enhance transcription elongation by preventing RNAP II backtracking, is critical both for basal HIV transcription and Tat-mediated transactivation. Thus, any model for the stimulatory effects of P-TEFb on HIV-1 transcription has to take into account the role of ELL2 and possibly several additional elongation factors.

A second completely unexpected finding to emerge from these studies is that Tat forms a stable complex with 7SK snRNP lacking HEXIM1 [76••]. Formation of Tat 7SK snRNP is likely mediated through direct interaction between Tat and 7SK RNA and is possibly plays an important role in HIV transcription by providing a pool of activated P-TEFb.

Conclusions

Devising strategies to eliminate this latent reservoir presents formidable challenges since the reservoir is established early during infection [77], is extremely stable, with an estimated half-life of 44 months [78], and can be replenished during episodes of viremia [79,80] or by homeostatic replacement of latently infected cells [81]. Since intensification of antiviral regimens has essentially no impact on eradicating the latent pool from the infected host [82], there have been recent calls to develop entirely novel forms of therapy to purge the pool of latent proviruses [83,84].

In the “shock and kill” strategy for HIV eradication [85,86], a “shock” phase is used to reactivate latent proviruses, and a “kill” phase is used to eliminate the induced cells through immune responses, viral cytopathogenicity or cytotoxic drugs. The detailed molecular studies described above strongly imply that effective activation of the entire latent viral pool may ultimately require a cocktail of drugs that stimulate both transcription initiation and P-TEFb mobilization.

The molecular studies reviewed here also emphasize that HIV has a plethora of potential drug vulnerabilities that could be exploited to prevent its re-emergence from latency. Although historically little progress has been made in finding selective inhibitors of Tat-activation of transcription the recent molecular and structural work on the Tat:P-TEFb complex suggest that the time is ripe to look for inhibitors of this critical interaction. Additionally there may be important opportunities to target the unique initiation complexes created by NF-κB and NFAT on the viral LTR.

It is a tribute to the success of molecular investigations into HIV transcription control and latency that the dream of achieving a functional cure for HIV infections, based devising new approaches to purge the latent reservoir, has now become a well-defined and realistic long-term research goal.

Keypoints

- HIV latency arises when levels of the regulatory protein Tat fall to below threshold levels.
- Transcriptional blocks restricting Tat levels are the result of epigenetic silencing, and in some cases, transcriptional interference by host promoters.
- Resting T-cells maintain latent proviruses by sequestering the Tat cofactor P-TEFb and the initiation factors NF-κB and NFAT in the cytoplasm.
- It is unlikely that purging of the latent viral pool can be achieved without activating HIV transcriptional initiation and elongation.

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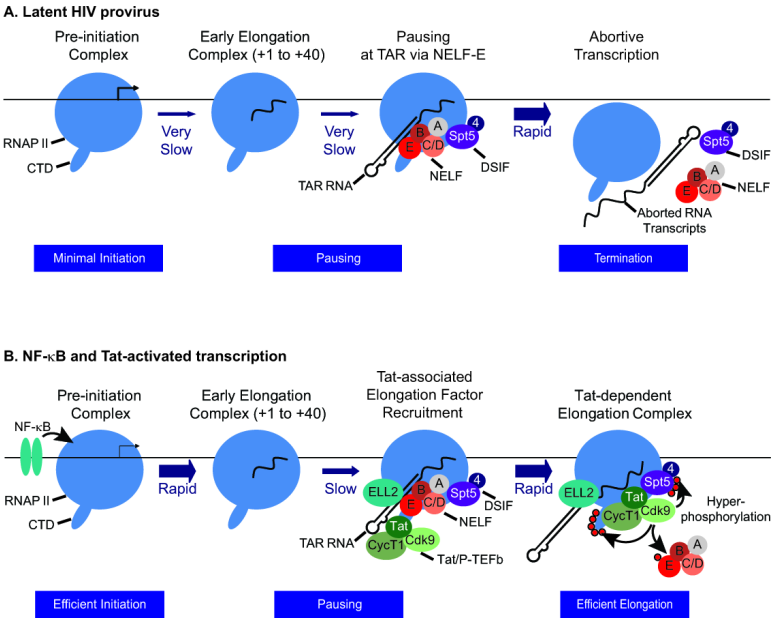

Figure 1. Reactivation of latent proviruses

(A) Latent HIV provirus. In latent proviruses transcription elongation is very inefficient due to absence of the transcription elongation factor NF-κB as well as chromatin restrictions (not shown for simplicity). The small number of transcription complexes that are able initiate and elongate through TAR are subject to additional elongation restrictions by NELF. NELF forces premature termination leading to an exceptionally low level of transcription from the latent provirus. (B) NF-κB and Tat-activated transcription. Initiation is strongly induced by NF-κB, which acts primarily to remove chromatin restrictions near the promoter through recruitment of histone acetyltransferases. Under these circumstances promoter clearance is also much more efficient and relatively few of the elongation complexes pause near the promoter. After the transcription through the TAR element, both NELF and the Tat/P-TEFb complex (including CDK9 and CycT1 and the accessory elongation ELL2) are recruited to the elongation complex via binding interactions with TAR RNA. This activates the CDK9 kinase and leads to hyperphosphorylation of the CTD of RNA polymerase II, Spt5 and NELF-E. The phosphorylation of NELF-E leads to its release. The presence of hyperphosphorylated RNAP II and Spt5 allows enhanced transcription of the full HIV genome.

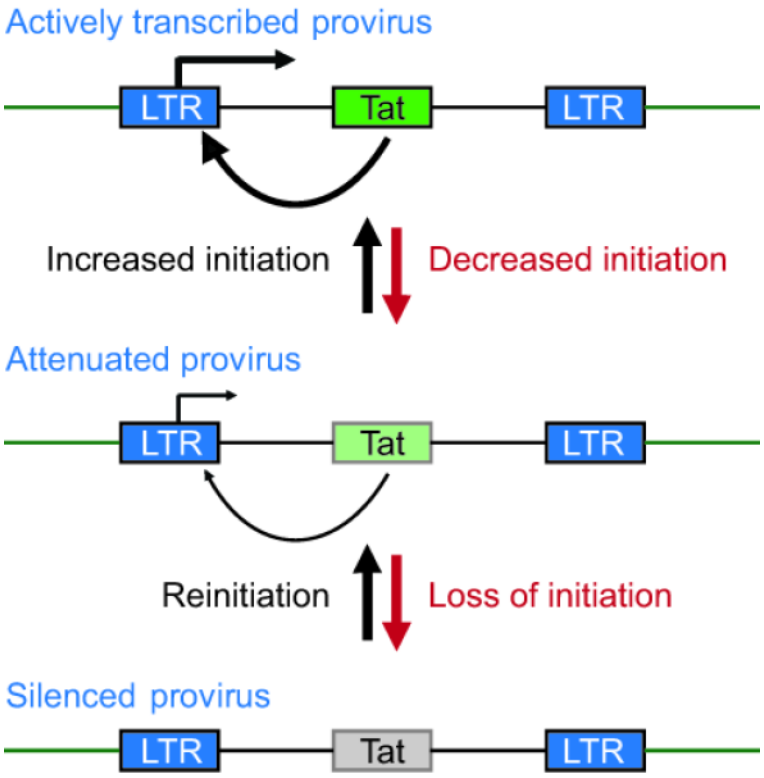

Figure 2. Autoregulation of HIV transcription by Tat

Small in initiation efficiency, due to transcriptional interference or epigenetic silencing, reduce Tat levels in the cell and disproportionately inhibit transcription, driving the HIV provirus into latency. Reinitiation stimulates Tat production and restores full transcription efficiency.

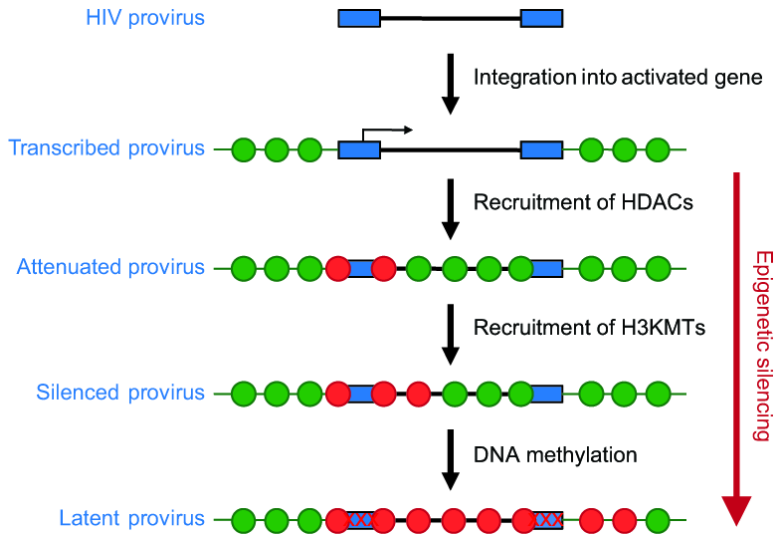

Figure 3. Stages in epigenetic silencing

Latent HIV proviruses almost invariably have deacteylated histones, but clones show heterogenous levels of methylated histones and methylated DNA. This suggests that silencing is an ordered and progressive process, with DNA methylation being a sign of the most repressed state.

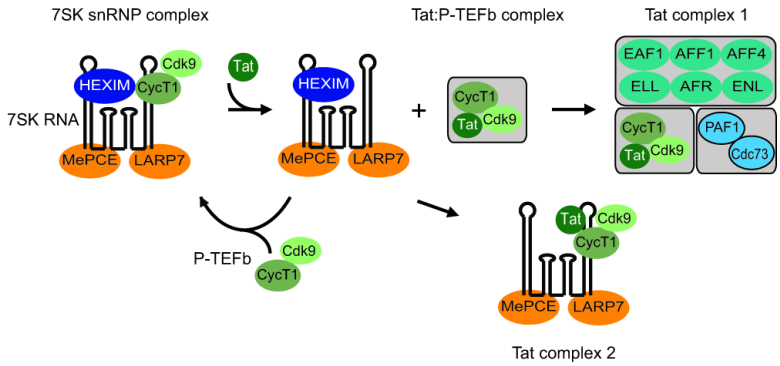

Figure 4. Control of P-TEFb by Tat

In resting CD4+ T-cells the majority of the P-TEFb in cells is found in a transcriptionally inactive snRNP complex containing 7SK RNA, HEXIM and the RNA binding proteins MePCE and LARP7. Tat disrupts this complex by displacing HEXIM and forming a stable complex with P-TEFb. Prior to recruitment to the transcription complex a larger complex is formed between P-TEFb and transcription elongation factors from the mixed lineage leukemia (MLL) family, including ELL2. A small fraction of the Tat in cell is also able to form a complex with 7SK RNA in the absence of HEXIM1.